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16. ABSTRACT

Male and female CD-1 mice were exposed to 2,4-dichlorophenol (DCP) in drinking water containing 10% emulphor for 90 days at concentrations of 0.2, 0.6, and 2.0 mg/ml. The mean daily consumption values ranged from 46.3 to 542 mg/kg/day for females and 34.9 and 419 mg/kg/day in males. In both sexes, water consumption was lower in the emulphor control group than in the water controls. Female mice tended to consume more of the solutions containing DCP than the vehicle control. There were no significant difference in body weight gain between females in the vehicle control and experimental groups. In males the rate of increase was lower in 2 of 14 weeks at the mid-dose and 4 of 14 weeks at the high dose. No differences were found in terminal organ weights or organ weight ratios in either sex. The only hematological differences were an increase in leukocytes at the high dose and an increase in polymorphonuclears at the low dose in males. Clinical chemistry parameters were altered in females only and include a decrease in creatinine (low dose), an increase in BUN/creatinine ratio (mid dose) and an increase in ALP (high dose). In an assessment of the hepatic microsomal mixed function oxidase system, no significant differences were found in the components in the system, component activity, the kinetics of ethylmorphine N-demethylase or the metabolism of testosterone. DCP, did not elicit consistent compound related or dose dependent significiant biological effects.

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Acute and Subchronic Toxicity of 2,4-Dichlorophenol in CD-1 Mice[1]

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Acute and Subchronic Toxicity of 2,4-Dichlorophenol in CD-1 Mice. BORZELLECA, J. F., HAYES, J. R., CONDIE, L. W., AND EGLE, J. L. JR. (1985) *Fundam. Appl. Toxicol.* 5, 478–486. The acute oral LD50 of 2,4-dichlorophenol (2,4-DCP) employing corn oil as vehicle was determined to be 1352 mg/kg for female and 1276 mg/kg for male CD-1 mice. CD-1 mice of both sexes were exposed to 2,4-DCP in drinking water containing 10% Emulphor for 90 days at concentrations of 0.2, 0.6, and 2.0 mg/ml providing theoretical daily doses of 50, 150, and 500 mg/kg. These concentrations resulted in mean daily doses of 50, 143, and 491 mg/kg for females and 40, 114, and 383 mg/kg for males. In both sexes, fluid consumption was lower in the vehicle control group (10% Emulphor) and in the 2,4-DCP treated groups than in the naive controls (deionized water). There were no biologically significant differences in body weight gain between females or males in the vehicle control and experimental groups. No differences were found in terminal organ weights or organ weight ratios in either sex. Hematological differences were observed in males only and included an increase in leukocytes (high dose) and an increase in polymorphonuclears (low dose). Clinical chemistry parameters were altered in females only and included a decrease in creatinine (low dose), an increase in BUN/creatinine ratio (mid dose), and an increase in ALP (high dose). In an assessment of the hepatic microsomal mixed function oxidase system, no significant differences were found in the components of the system, component activity, the kinetics of ethylmorphine *N*-demethylase, or the metabolism of testosterone. Administration of 2,4-DCP to CD-1 mice of both sexes for 90 days at mean doses of 40 to 491 mg/kg/day in drinking water did not elicit either consistent compound-related or dose-dependent significant toxicological effects. © 1985 Society of Toxicology.

2,4-Dichlorophenol (2,4-DCP) is used in the manufacture of herbicides, germicides, temporary soil sterilants, seed disinfectants, plant growth regulators, mothproofing agents, miticides, and wood preservatives (Holcomb *et al.*, 1982). The compound has been identified in drinking water and concern has been expressed over possible adverse health effects (NAS, 1977).

Acute oral LD50 values for 2,4-DCP have been reported as 580 mg/kg (Christensen *et al.*, 1976), 2830 mg/kg (Vernot *et al.*, 1977), and 4000 mg/kg (Kobayashi *et al.*, 1972) for the rat; 1600 mg/kg (Kobayashi *et al.*, 1972) and 1630 mg/kg (Vernot *et al.*, 1977) for the mouse. Acute intraperitoneal and subcutaneous LD50 values for the rat have been reported to be 430 and 1730 mg/kg, respectively (Christensen *et al.*, 1976).

In studies of the toxicity of 2,4-DCP to aquatic species, Hattula *et al.* (1981) reported a 24-hr LC50 of 1.7 ppm (mg/liter) for trout, Buccafusco *et al.* (1981) found LC50 values for bluegill of 4.7 mg/liter (24 hr) and 2.0 mg/liter (96 hr), and Holcombe *et al.* (1982) determined that the survival of fathead minnows significantly decreases after 28 days of

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exposure to 2,4-DCP concentrations of 0.46 mg/liter and above. In a mutagenicity study, Probst *et al.* (1981) concluded that 2,4-DCP was negative in both the hepatocyte unscheduled DNA synthesis and modified Ames assays.

Longer term toxicity has been studied by Kobayashi *et al.* (1972). Male mice received 2,4-DCP as a dietary admixture for 6 months at doses of 45, 100, and 230 mg/kg/day. The only suggestion of toxicity was a slight abnormality in liver histopathology in mice receiving the highest dosage.

The present investigation was undertaken to study the effects of subchronic administration of 2,4-DCP in drinking water at concentrations expected to produce a daily intake higher than the Kobayashi study.

METHODS

Animals and housing. Male and female CD-1 mice were obtained at 6 weeks of age from Charles River Breeding Laboratories, Wilmington, Massachusetts. After a 4-day quarantine, the mice were randomized and housed individually in plastic shoebox cages with hardwood bedding (PWI Hardwood Sawdust, Lowville, N.Y.) in an animal room maintained at 21–24°C and 40–60% relative humidity with light–dark cycles of 12 hr duration. The mice were caged vertically to minimize light and temperature variations between exposure groups. Purina Rodent Chow No. 5001 (Ralston Purina Co., St. Louis, Mo.) and the various drinking solutions were available *ad libitum*.

Test solutions. 2,4-DCP (Lot 2022HJ) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. The identity and purity (99%) were confirmed prior to initiation of the study. Preliminary studies indicated that 2,4-DCP solutions were unpalatable and Emulphor, a polyoxyethylated vegetable oil (GAF Corp., Linden, N.J.), was added to the drinking solutions to increase palatability and solubility. DCP was found to be stable for at least 5 days in 10% Emulphor. Solutions were prepared twice weekly in (1:9 v/v) Emulphor:water. Concentrations of 2,4-DCP were 0.2, 0.6, and 2.0 mg/ml (the limit of solubility).

Acute toxicity. 2,4-DCP was dissolved in corn oil and administered by gavage. Concentrations were varied to permit a constant volume (10 ml/kg) of administration. Five dose levels were employed with eight mice of each sex per dosage group. The animals were fasted for 18 hr prior to dosing with food being returned 1 hr after dosing. The mice were continuously observed for 1 hr after dosing, hourly for the next 8 hr and then twice daily for the next 14 days. All mice that died during the observation period and those that survived for 14 days were necropsied. The LD50 was computed by log probit analysis as described by Finney (1971).

Subchronic toxicity. A total of 200 mice (20 of each sex) were exposed to each of the following drinking water solutions for a period of 90 days: naive control (deionized water), vehicle control (10% Emulphor), and 2,4-DCP at concentrations of 0.2, 0.6, and 2.0 mg/ml.

All solutions were available *ad libitum* throughout the study. The drinking solutions were changed twice each week and consumption was measured at each change. Animals were weighed on the first day of dosing and weekly thereafter. Mice were observed twice daily, with at least 5 hr between observations, for changes in health status. Those that either died or were judged to be moribund and sacrificed, were necropsied, as well as those that survived the 90-day treatment. The following parameters were measured at the conclusion of the study.

Necropsy, hematology, and clinical chemistry. Terminal body weights and organ weights (brain, liver, spleen, lungs, thymus, and kidneys) were determined at necropsy. Tissue samples from the above organs and others were preserved and saved in neutral buffered formalin. Leukocyte, erythrocyte, and platelet counts were made with a Model ZBI Coulter counter. Hematocrits were determined and hemoglobin was assayed as cyanohemoglobin. Leukocyte differentials were evaluated by the Wright–Giemsa staining procedure. Plasma extrinsic coagulation activity was determined by prothrombin time and intrinsic coagulation activity by activated partial thromboplastin time, employing reagents from General Diagnostics, Morris Plains, New Jersey. Plasma fibrinogen levels were determined using reagents from Dade Diagnostics Inc., Miami, Florida, following the procedures recommended by the manufacturer.

Clinical chemistry determinations were made using a Model 200 Abbott bichromatic analyzer. Serum sodium and potassium concentrations were determined using a flame photometer and chloride determined using a chloridometer. Other parameters measured were SGPT, SGOT, LDH, ALP, BUN, glucose, bilirubin, albumin, total protein, cholesterol, creatinine, phosphorous, and calcium. Calculated values included globulin and albumin/globulin ratio.

Hepatic microsomal enzyme activity. Microsomes were isolated as previously described (Hayes *et al.*, 1973). Protein content was determined by the method of Lowry *et al.* (1951). The method of Omura and Sato (1964) was used for the determination of cytochrome *P*-450 employing an Aminco DW-2 dual-wavelength scanning spectrophotometer. Ethylmorphine *N*-demethylase kinetics were determined using a procedure modified from Davies *et al.* (1968). Reaction rates were linear with both time and protein concentration. Kinetic constants were

TABLE 1

ACUTE ORAL TOXICITY OF 2,4-DICHLOROPHENOL IN MALE AND FEMALE CD-1 MICE

Sex	N	LD10[a]	LD50[a]	LD90[a]	Slope function[b]	Probit of slope[b]
Male	40	710 (386–936)	1276 (982–1569)	2293 (1825–3595)	1.606	1.265
Female	40	772 (481–977)	1352 (1094–1670)	2370 (1874–3795)	1.574	1.252

[a] Values in mg/kg (95% confidence limit).
[b] Slope function = (LD85/LD50 + LD50/LD10)/2.
[c] Log probit slope computed by SAS Probit Analysis for LD50.

calculated employing the computer program devised by Cleland (1963). Ethoxycoumarin O-deethylase activity was determined by the method of Duppel and Ullrich (1976). The reaction was allowed to run for 10 min at 25° at which time the relative fluorescence of the product (7-hydroxycoumarin) was determined in an Aminco–Bowman spectrofluorimeter. An excitation wavelength of 360 nm and an emission wavelength of 450 nm were utilized. Testosterone hydroxylation was determined by the method of Conney and Klutch (1963). Cytochrome *P*-450 reductase activity was determined by the method of Peterson *et al.* (1978) employing a DW-2 dual-wavelength spectrophotometer in the dual-wavelength mode and employing an Aminco–Morrow stopped-flow device. High-speed data aquisition was carried out employing a Bascon-Turner high-speed data acquisition recorder. Cytochrome *c* reductase activity was determined essentially as described by Gigon *et al.* (1969) except that the reaction was carried out employing the stopped-flow spectrophotometer to ensure the determination of the initial velocity.

Statistical analysis. Results were analyzed using Bartlett's test for homogeneity, parametric ANOVA, and Dunnett's multirange test. In cases where Bartlett's test indicated nonhomogeneity, the nonparametric method of Wilcoxon was employed. Those values which differed from the vehicle control group at $p \leq 0.05$ were considered significant.

RESULTS

Acute Toxicity

The LD50 values in milligrams per kilogram with 95% confidence limits were 1276 (982–1569) for males and 1352 (1094–1670) for females (Table 1). These values are somewhat lower, but in the range previously reported (Kobayashi *et al.*, 1972; Vernot *et al.*, 1977). All animals displayed signs of intoxication within 10–15 min after dosing. Ataxia, loss of righting reflex, slight tremors, salivation, labored breathing, and depression were observed. All deaths occurred within 24 hr; most deaths occurred within 6 hr. Severity of signs and times of death were dose dependent. Animals found dead were immediately necropsied and those that survived the 14-day observation period were sacrificed and necropsied. No gross pathological lesions were evident either externally or internally at any dosage level.

Subchronic Toxicity

Fluid consumption. Mean daily fluid consumption, expressed as milliters per kilogram per day, is shown in Table 2. Mean values

TABLE 2

MEAN FLUID CONSUMPTION (ml/kg/day) FOR CD-1 MICE EXPOSED TO 2,4-DICHLOROPHENOL FOR 90 DAYS

Concentration	Female	Male
Naive	303 ± 5*	226 ± 4*
Emulphor (10%)	219 ± 5	202 ± 3
0.2 mg/ml	247 ± 3	200 ± 3
0.6 mg/ml	238 ± 3	190 ± 3
2.0 mg/ml	245 ± 4	191 ± 2

Note. Values are means ± SE.
* Differ significantly ($p \leq 0.05$) from the 10% Emulphor group.

for both female and male naive control mice were significantly higher than the respective vehicle control throughout the study. Weekly water consumption (data not shown) indicated that the addition of 2,4-DCP to the drinking water resulted in increased water consumption during certain weeks in females. Weekly water consumption of males did not indicate a consistent trend.

Test material consumption. The concentrations of 2,4-DCP employed were selected with the intention of providing doses of 50, 150 and 500 mg/kg/day. Actual mean daily consumption values of 2,4-DCP in milligrams per kilogram per day are displayed in Table 3. The theoretical doses are used in the tables summarizing the results to allow presentation of data from both sexes on single tables.

Body weights. Mean increases in body weight (grams) at the end of 14 weeks for female mice were: naive control (2.6), vehicle control (5.5), low dose (5.4), mid dose (4.0), and high dose (5.3). None of the values differed significantly from vehicle control. However, naive control values were significantly lower at Weeks 6 and 7 and 10–13. For male mice the mean increases in body weight at 14 weeks were: naive control (2.2), vehicle control (5.6), low dose (4.7), mid dose (5.0), and high dose (5.1). The increase in the naive control group was significantly greater at Week 2 and significantly less at Weeks 8 and 10–13. These results indicate that Emulphor may produce small increases in body weight.

TABLE 3

MEAN CONSUMPTION OF TEST MATERIAL (mg/kg/day) FOR CD-1 MICE EXPOSED TO 2,4-DICHLOROPHENOL FOR 90 DAYS

Concentration	Female	Male
Naive	—	—
Emulphor (10%)	—	—
0.2 mg/ml	50 ± 1	40 ± 1
0.6 mg/ml	143 ± 2	114 ± 2
2.0 mg/ml	491 ± 8	383 ± 5

Note. Values are means ± SE.

Terminal body and organ weights. Terminal body and organ weights are summarized in Table 4. No 2,4-DCP-associated alteration in terminal body weight was detectable.

There were no 2,4-DCP-induced alterations in absolute organ weights or when expressed as brain and body weight ratios. This finding is consistent with the lack of any evidence of gross pathology during necropsy. Absolute and relative liver and kidney weights were significantly decreased, and brain weight significantly increased in both sexes in the naive control group as compared to the Emulphor control. This indicates that Emulphor is not without effect in this study.

Hematology. Hematology and differential cell count values are shown in Table 5. No significant differences were found in female mice receiving 2,4-DCP. The hematocrit was lower in the male naive control group. Males demonstrated significantly elevated leukocyte levels in the high dose group. Differential leukocyte counts included significantly higher polymorphonuclears in males in the low dose and naive control groups.

Clinical chemistry. Clinical chemistry values or ratios are summarized in Table 6. In females the only significant differences observed in the treated groups were a decrease in creatinine (low dose), an increase in the BUN/creatinine ratio (mid dose), and an increase in ALP (high dose). In the naive control group there was an increase in the BUN/creatinine ratio and decreases in cholesterol, albumin, calcium, and creatinine. In males, the only significant differences were in the naive controls and involved decreases in ALP, calcium, and creatinine and an increased BUN/creatinine ratio.

Mixed function oxidase system. Studies conducted to determine if 2,4-DCP affects components of the hepatic *P*-450 dependent monoxygenase system at the concentrations employed are summarized in Table 7. Cytochrome P-450 levels were significantly lower in the male naive control mice, and ethoxy-

TABLE 4

TERMINAL BODY AND ORGAN WEIGHTS (mg) OF CD-1 MICE EXPOSED TO 2,4-DCP FOR 90 DAYS

	Naive	Emulphor (10%)	DCP (mg/kg) 50	150	500
			Females		
N	20	20	20	19	18
Body wt (g)	30.9 ± 0.5	31.5 ± 0.6	32.1 ± 0.6	31.6 ± 0.1	30.5 ± 0.9
Brain	510 ± 10*	470 ± 10	480 ± 10	480 ± 10	470 ± 10
(% body wt)	1.6 ± 0.0*	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.0
Liver	1670 ± 50*	2320 ± 70	2480 ± 100	2290 ± 80	2350 ± 90
(% body wt)	5.4 ± 0.1*	7.3 ± 0.1	7.7 ± 0.2	7.2 ± 0.2	7.8 ± 0.3
(% brain wt)	327 ± 70*	493 ± 15	518 ± 28	473 ± 16	497 ± 17
Spleen	100 ± 5	90 ± 5	100 ± 4	100 ± 5	100 ± 5
(% body wt)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
(% brain wt)	19 ± 1	20 ± 1	20 ± 0.1	21 ± 0.0	20 ± 0.0
Lungs	210 ± 10	210 ± 10	220 ± 10	200 ± 10	200 ± 10
(% body wt)	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
(% brain wt)	41 ± 2	44 ± 2	45 ± 2	42 ± 2	42 ± 2
Thymus	40 ± 3	40 ± 3	30 ± 4	40 ± 3	40 ± 5
(% body wt)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
(% brain wt)	7 ± 0.1*	9 ± 0.1	7 ± 1	8 ± 1	8 ± 1
Kidneys	430 ± 10*	490 ± 13	520 ± 10	490 ± 20	490 ± 10
(% body wt)	1.4 ± 0.0*	1.1 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0
(% brain wt)	85 ± 2*	104 ± 3	105 ± 3	102 ± 4	103 ± 2
			Males		
N	20	20	20	19	20
Body wt (g)	38.6 ± 0.5	39.9 ± 0.7	40.4 ± 0.6	39.8 ± 0.7	38.7 ± 0.5
Brain	500 ± 10*	490 ± 20	490 ± 10	500 ± 30	490 ± 10
(% body wt)	1.3 ± 0.0*	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.1	1.3 ± 0.0
Liver	2080 ± 60*	3090 ± 110	3350 ± 90	3070 ± 100	3060 ± 70
(% body wt)	5.4 ± 0.1*	7.7 ± 0.2	8.3 ± 0.2	7.6 ± 0.2	7.9 ± 0.1
(% brain wt)	420 ± 10*	640 ± 30	690 ± 20	620 ± 30	630 ± 10
Spleen	80 ± 4	90 ± 5	90 ± 5	90 ± 6	89 ± 4
(% body wt)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
(% brain wt)	17 ± 1	19 ± 1	19 ± 1	19 ± 1	18 ± 1
Lungs	220 ± 7	230 ± 7	240 ± 10	240 ± 6	240 ± 9
(% body wt)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
(% brain wt)	44 ± 2	48 ± 2	50 ± 3	49 ± 2	49 ± 2
Thymus	40 ± 3	40 ± 3	40 ± 4	40 ± 2	36 ± 2
(% body wt)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
(% brain wt)	7.7 ± 0.5	8.0 ± 0.5	8.1 ± 0.8	7.0 ± 0.5	7.5 ± 0.5
Kidneys	650 ± 20*	740 ± 10	740 ± 10	720 ± 20	720 ± 10
(% body wt)	1.7 ± 0.0*	1.9 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
(% brain wt)	130 ± 4*	150 ± 5	150 ± 4	150 ± 5	150 ± 3

Note. Values are means ± SE. *N* = number of live animals in each group at the conclusion of the study.
* Differs significantly ($p \leq 0.05$) from the 10% Emulphor group.

TABLE 5

HEMATOLOGY AND DIFFERENTIAL CELL COUNT VALUES FOR CD-1 MICE EXPOSED TO 2,4-DCP FOR 90 DAYS

Parameter	Sex	Naive	Emulphor (10%)	DCP (mg/kg) 50	150	500
Hemoglobin (g%)	F	13.4 ± 0.3	13.3 ± 0.4	13.7 ± 0.2	14.3 ± 0.2	14.1 ± 0.2
	M	13.8 ± 0.2	14.1 ± 0.3	13.6 ± 0.2	13.9 ± 0.2	14.2 ± 0.2
Hematocrit (%)	F	38.0 ± 0.7	38.3 ± 1.0	38.0 ± 0.4	39.8 ± 0.7	39.4 ± 0.5
	M	38.8 ± 0.5*	40.7 ± 0.7	38.9 ± 0.4	39.1 ± 0.4	39.7 ± 0.6
Leukocytes (10^3/mm^3)	F	8.2 ± 0.6	7.2 ± 0.8	7.6 ± 0.5	8.8 ± 0.9	9.5 ± 1.2
	M	7.1 ± 0.6	6.9 ± 0.4	6.5 ± 0.3	8.0 ± 0.5	9.6 ± 0.5*
Erythrocytes (10^6/mm^3)	F	8.1 ± 0.2	7.9 ± 0.2	8.1 ± 0.2	8.4 ± 0.3	8.4 ± 0.2
	M	8.6 ± 0.2	8.6 ± 0.2	8.6 ± 0.1	8.6 ± 0.2	8.6 ± 0.2
Platelets (10^5/mm^3)	F	5.4 ± 0.3	5.7 ± 0.4	5.5 ± 0.2	4.8 ± 0.3	5.0 ± 0.4
	M	5.7 ± 0.2	5.1 ± 0.3	5.6 ± 0.4	5.4 ± 0.2	5.6 ± 0.3
Prothrombin time (sec)	F	14.9 ± 1.2	13.6 ± 0.5	13.8 ± 1.3	14.6 ± 1.2	15.4 ± 0.9
	M	14.1 ± 0.7	14.8 ± 0.9	14.3 ± 0.5	14.2 ± 0.9	14.9 ± 1.1
Fibrinogen (mg%)	F	157 ± 6	155 ± 11	167 ± 8	164 ± 5	156 ± 11
	M	191 ± 4	176 ± 7	173 ± 5	175 ± 4	169 ± 6
Lymphocytes	F	85.9 ± 1.4	86.8 ± 1.6	84.0 ± 2.4	89.1 ± 2.0	87.0 ± 3.0
	M	79.9 ± 3.8	89.1 ± 1.8	85.8 ± 2.1	88.4 ± 1.9	86.6 ± 3.4
Polymorpho-nuclears	F	5.9 ± 1.2	6.2 ± 0.9	7.4 ± 1.2	5.4 ± 1.9	9.2 ± 2.4
	M	9.3 ± 2.2*	4.6 ± 0.8	10.0 ± 1.7*	7.6 ± 1.8	9.3 ± 3.0
Monocytes	F	7.9 ± 1.4	6.5 ± 1.3	8.3 ± 1.8	5.0 ± 0.9	3.1 ± 1.3
	M	6.1 ± 1.4	6.0 ± 1.5	3.7 ± 1.1	3.8 ± 1.1	3.9 ± 1.5
Eosinophils	F	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
	M	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.3

Note. Values are means ± SE.
* Differs significantly ($p \leq 0.05$) from the 10% Emulphor group.

coumarin *O*-deethylase activity was greater in the female naive group. No toxicologically significant compound related effects on the parameters measured were observed.

DISCUSSION

Emulphor, used as a vehicle intended to enhance the solubility and palatability of 2,4-DCP in water, apparently exerted an organoleptic effect. Addition of 2,4-DCP somewhat overcame this taste aversion in the females, but not in the males. Consumption of fluid was sufficiently high to yield daily intake values considerably higher than the maximum of 230 mg/kg in the Kobayashi *et al.* (1972) study, without evidence of toxicity. Female mice in the high concentration group received twice that amount.

Assessment of the results of this study reveals very few significant differences between the vehicle group and those receiving 2,4-DCP. There were no significant differences in organ weights or organ weight ratios. There were no toxicologically significant alterations in serum enzyme or electrolyte levels and hematology in male animals and the few changes seen in females do not appear to be treatment related. There was no evidence of any effect of 2,4-DCP on the components or activity of the hepatic mixed function oxidase system.

TABLE 6

CLINICAL CHEMISTRY VALUES OF CD-1 MICE EXPOSED TO 2,4-DCP FOR 90 DAYS

Parameter	Sex	Naive	Emulphor (10%)	DCP (mg/kg) 50	150	500
LDH (IU/liter)	F	1317 ± 272	1316 ± 226	1211 ± 160	1145 ± 70	1171 ± 204
	M	867 ± 113	907 ± 140	915 ± 72	952 ± 107	838 ± 37
SGPT (IU/liter)	F	46 ± 7	73 ± 21	69 ± 23	57 ± 9	87 ± 30
	M	37 ± 2	44 ± 4	55 ± 10	75 ± 15	48 ± 8
SGOT (IU/liter)	F	131 ± 22	140 ± 35	179 ± 42	147 ± 29	136 ± 29
	M	94 ± 12	78 ± 6	87 ± 15	97 ± 17	91 ± 17
ALP (IU/liter)	F	62 ± 4	72 ± 4	90 ± 18	127 ± 31	108 ± 14*
	M	58 ± 5*	100 ± 13	90 ± 5	114 ± 16	82 ± 6
Bilirubin (mg%)	F	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
	M	1.0 ± 0.2	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
BUN (mg%)	F	27.8 ± 1.3	27.6 ± 0.7	28.3 ± 0.7	30.6 ± 1.2	31.4 ± 3.2
	M	31.0 ± 2.4	30.9 ± 1.5	31.5 ± 1.2	29.7 ± 1.7	28.7 ± 1.4
Protein (g%)	F	5.2 ± 0.1	5.7 ± 0.2	5.6 ± 0.2	5.6 ± 0.1	5.7 ± 0.3
	M	6.1 ± 0.2	6.3 ± 0.2	6.2 ± 0.2	6.3 ± 0.2	5.8 ± 0.2
Glucose (mg%)	F	175 ± 12	171 ± 13	161 ± 9	160 ± 10	167 ± 11
	M	177 ± 11	199 ± 5	199 ± 10	183 ± 8	196 ± 9
Cholesterol (mg%)	F	101 ± 8*	141 ± 14	115 ± 9	111 ± 9	137 ± 7
	M	148 ± 10	141 ± 10	132 ± 10	132 ± 11	153 ± 14
Albumin (g%)	F	3.5 ± 0.1*	3.7 ± 0.1	3.4 ± 0.2	3.6 ± 0.1	3.5 ± 0.2
	M	3.2 ± 0.1	3.7 ± 0.2	3.9 ± 0.1	3.8 ± 0.2	3.7 ± 0.1
Globulin (g%)	F	1.7 ± 0.1	2.0 ± 0.2	2.3 ± 0.3	2.0 ± 0.2	2.3 ± 0.3
	M	2.9 ± 0.3	2.6 ± 0.3	2.4 ± 0.3	2.5 ± 0.2	2.1 ± 0.2
ALB/globulin ratio	F	2.2 ± 0.3	1.9 ± 0.2	1.6 ± 0.2	1.9 ± 0.2	1.8 ± 0.3
	M	1.3 ± 0.2	1.6 ± 0.2	1.9 ± 0.2	1.7 ± 0.2	1.9 ± 0.2
Phosphorus (mg%)	F	9.7 ± 0.4	10.7 ± 0.5	10.7 ± 0.5	11.0 ± 0.7	10.4 ± 0.5
	M	11.0 ± 0.7	11.8 ± 0.7	11.5 ± 0.4	11.7 ± 0.7	10.8 ± 0.3
Calcium (mg%)	F	10.3 ± 0.1*	11.5 ± 0.1	11.4 ± 0.3	11.0 ± 0.2	11.1 ± 0.2
	M	10.8 ± 0.2*	11.5 ± 0.1	11.8 ± 0.4	11.6 ± 0.1	11.3 ± 0.2
Sodium (meq/liter)	F	155 ± 1	156 ± 1	158 ± 1	157 ± 1	157 ± 1
	M	155 ± 1	158 ± 1	155 ± 2	157 ± 1	158 ± 1
Chloride (meq/liter)	F	108 ± 1	105 ± 1	108 ± 1	108 ± 1	109 ± 2
	M	108 ± 2	106 ± 2	104 ± 2	106 ± 1	105 ± 1
Potassium (meq/liter)	F	7.7 ± 0.4	8.4 ± 0.5	7.9 ± 0.4	8.0 ± 0.4	7.8 ± 0.5
	M	9.3 ± 0.6	8.9 ± 0.3	9.3 ± 0.4	8.8 ± 0.4	9.1 ± 0.5
Creatinine (mg%)	F	1.1 ± 0.1*	1.5 ± 0.1	1.2 ± 0.1*	1.3 ± 0.1	1.2 ± 0.1
	M	0.9 ± 0.1*	1.4 ± 0.1	1.4 ± 0.1	1.2 ± 0.0	1.2 ± 0.1
BUN/ creatinine ratio	F	25.9 ± 1.3*	19.3 ± 1.0	24.2 ± 2.2	24.9 ± 1.6*	22.4 ± 1.1
	M	34.7 ± 1.8*	22.7 ± 0.7	23.1 ± 1.4	24.2 ± 0.9	25.7 ± 2.7

Note. Values are means ± SE.

* Differs significantly ($p \leq 0.05$) from the 10% Emulphor group.

TABLE 7

EFFECT OF 90-DAY ADMINISTRATION OF 2,4-DCP TO CD-1 MICE ON THE COMPONENTS OF THE HEPATIC MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM AND ON COMPONENT ACTIVITY

Parameter	Sex	N	Naive	Emulphor (10%)	DCP (mg/kg) 50	150	500
Components							
Microsomal protein (mg/g liver)	F	6	28.5 ± 1.4	31.9 ± 0.7	31.3 ± 1.1	31.7 ± 1.1	31.9 ± 1.6
	M	6	26.5 ± 2.2	29.1 ± 2.6	27.8 ± 3.1	27.0 ± 2.8	29.0 ± 3.6
Cytochrome *P*-450 (nmol/mg protein)	F	5	0.39 ± 0.07	0.44 ± 0.03	0.46 ± 0.07	0.39 ± 0.11	0.54 ± 0.07
	M	5	0.34 ± 0.07*	0.59 ± 0.07	0.72 ± 0.15	0.57 ± 0.21	0.48 ± 0.24
Component activity							
NADPH–cytochrome *c* reductase (nmol reduced/ mg protein/min)	F	4	244 ± 43	182 ± 41	250 ± 39	218 ± 54	236 ± 34
	M	4	185 ± 12	220 ± 13	222 ± 8	220 ± 26	166 ± 19
NADPH–cytochrome *P*-450 reductase (nmol reduced/ mg protein/min)	F	4	27.7 ± 5.6	21.7 ± 4.1	29.3 ± 0.6	26.5 ± 3.3	28.7 ± 4.0
	M	4	35.9 ± 4.6	29.2 ± 2.3	30.9 ± 7.0	27.1 ± 2.2	39.2 ± 12.0
Ethylmorphine *N*-demethylase							
K_m (mM)	F	5	0.39 ± 0.09	0.52 ± 0.09	0.53 ± 0.10	0.32 ± 0.08	0.32 ± 0.07
	M	5	0.38 ± 0.08	0.49 ± 0.09	0.58 ± 0.09	0.60 ± 0.05	0.49 ± 0.08
V_{max} (nmol CH_2O produced/ mg protein/min)	F	5	4.30 ± 1.37	4.50 ± 0.59	5.71 ± 0.88	5.37 ± 0.58	5.28 ± 1.49
	M	5	5.71 ± 1.04	8.25 ± 2.15	7.01 ± 0.80	9.12 ± 1.00	7.82 ± 1.62
Ethoxycoumarin *o*-deethylase (nmol product/mg protein min × 10^{-3})	F	5	132 ± 3*	86 ± 6	70 ± 10	86 ± 10	86 ± 20
	M	5	96 ± 2	73 ± 2	82 ± 14	94 ± 50	73 ± 6
Testosterone metabolism (pmol/mg protein/min)							
6β-Hydroxytestosterone	F	3	182.5 ± 50.0	150.0 ± 38.0	82.5 ± 45.0	92.5 ± 30.0	150.5 ± 55.0
	M	3	150.0 ± 38.0	107.3 ± 19.8	108.7 ± 31.2	102.5 ± 42.5	105.0 ± 10.0
7α-Hydroxytestosterone	F	3	52.5 ± 20.0	40.0 ± 10.0	27.5 ± 10.0	30.0 ± 7.5	42.5 ± 7.5
	M	3	47.5 ± 17.5	44.2 ± 11.6	32.7 ± 3.9	30.0 ± 5.0	32.5 ± 2.5
16α-Hydroxytestosterone	F	3	40.0 ± 10.0	50.0 ± 15.0	25.0 ± 7.5	35.0 ± 17.5	45.0 ± 2.5
	M	3	47.5 ± 10.0	46.6 ± 11.5	33.4 ± 6.2	35.0 ± 5.0	32.5 ± 2.5

Note. Values are means ± SE.
* Differs significantly ($p \leq 0.05$) from the 10% Emulphor group.

It is concluded that average daily consumption of as much as 350–540 mg/kg of 2,4-DCP in drinking water by mice for 90 days did not result in toxicologically significant alterations in the parameters investigated.

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